

What is claimed is:

1. An E1-complementing cell line useful for production of recombinant E1-defective adenoviruses in the absence of detectable replication-competent adenovirus, said E1-complementing cell line comprising an aneuploid cell line stably transformed with a nucleic acid molecule comprising nucleic acid sequences encoding adenovirus E1a and adenovirus E1b under the control of a phosphoglycerate kinase (PGK) promoter, and wherein the nucleic acid sequences further comprise a deletion of adenovirus sequences 5' to the sequences encoding adenovirus E1a.
2. The E1-complementing cell line according to claim 1, wherein the aneuploid cell line is a HeLa cell line.
3. The E1-complementing cell line according to claim 1, wherein the nucleic acid sequences further comprise nucleic acid sequences of the pIX gene region.
4. The E1-complementing cell line according to claim 1, wherein the nucleic acid molecule is a plasmid vector.
5. The E1-complementing cell line according to claim 1, wherein the nucleic acid molecule comprises multiple copies of the sequences encoding adenovirus E1a and adenovirus E1b.
6. The E1-complementing cell line according to claim 1, wherein the E1-complementing cell line comprises multiple copies of said nucleic acid molecule.
7. The E1-complementing cell line according to claim 1, wherein the sequences encoding adenovirus E1a and the sequences encoding E1b are independently selected from adenovirus type 5.

8. The E1-complementing cell line according to claim 1, wherein the cell line is selected from the group consisting of GH364 and GH354.

9. An adenovirus E1-complementing cell line designated GH329, deposited with the ATCC under accession number PTA-803.

10. A method for packaging of E1-defective adenoviral particles in the absence of replication competent adenovirus, said method comprising the steps of:

(a) providing cells from an E1-complementing cell line comprising an aneuploid cell line stably transformed with a nucleic acid molecule comprising nucleic acid sequences encoding adenovirus E1a and adenovirus E1b under the control of a phosphoglycerate kinase (PGK) promoter, wherein the nucleic acid sequences further comprise a deletion of adenovirus sequences 5' to the sequences encoding adenovirus E1a;

(b) transfecting said cells with a recombinant vector comprising, from 5' to 3', adenovirus 5' inverted terminal repeat sequences (ITRs), nucleic acid sequences encoding adenovirus pIX under the control of sequences which direct expression of adenovirus pIX in said cells, and a defect in the adenovirus E1 region, and adenovirus 3' ITRs; and

(c) culturing said transfected cells under conditions which permit packaging of the E1-defective vector into a recombinant E1-defective adenoviral particle.

11. The method according to claim 10, wherein said recombinant vector further comprises a selected transgene.

12. The method according to claim 11, wherein said transgene is located between the 5' and 3' ITRs.

13. The method according to claim 10, further comprising the step of transfecting said cells with a second recombinant vector comprising adenovirus sequences encoding at least one adenoviral gene and a defect in the adenovirus E1 region.

14. The method according to claim 13, wherein said second recombinant vector encodes adenovirus E2a.

15. The method according to claim 13, wherein said second recombinant vector encodes adenovirus E4 or a functional fragment thereof.

16. The method according to claim 15, wherein the functional fragment is E4 ORF6.

17. The method according to claim 10, wherein the E1-complementing cell line is selected from the group consisting of GH329, ATCC PTA-803; GH364 and GH354.

18. A method of amplifying E1-defective adenoviral particles in the absence of replication competent adenovirus, the method comprising the steps of:

(a) infecting an E1-complementing cell line with E1-defective adenoviruses, wherein said cell line comprises an aneuploid cell line stably transformed with a nucleic acid molecule comprising nucleic acid sequences encoding adenovirus E1a and adenovirus E1b under the control of a phosphoglycerate kinase (PGK) promoter, and wherein the nucleic acid sequences further comprise a deletion of adenovirus sequences 5' to the sequences encoding adenovirus E1a;

(b) passaging the E1-defective adenoviral particles on the E1-complementing cell line for 2 to 20 passages, and

(c) collecting the E1-defective adenoviral particles.

19. The method according to claim 18, wherein the E1-defective adenoviruses of (a) are prepared by the steps comprising:

(i) providing cells from an E1-complementing cell line comprising an aneuploid cell line stably transformed with a nucleic acid molecule comprising nucleic acid sequences encoding adenovirus E1a and adenovirus E1b under the control of a phosphoglycerate kinase (PGK) promoter, wherein the nucleic acid sequences further comprise a deletion of adenovirus sequences 5' to the sequences encoding adenovirus E1a;

(ii) transfecting said cells with a recombinant vector comprising adenovirus 5' and 3' inverted terminal repeat sequences (ITRs), nucleic acid sequences encoding adenovirus pIX under the control of sequences which direct expression of adenovirus pIX in said cells, and a defect in the adenovirus E1 region;

(iii) culturing said transfected cells under conditions which permit packaging of the E1-defective vector into a recombinant E1-defective adenoviral particle; and

(iv) purifying the recombinant E1-defective adenoviral particle from substantially all cellular debris.